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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/522,405	09/30/2005	Andrea Cossarizza	28902.0015	5546

30827 7590 11/03/2006

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EXAMINER
STAPLES, MARK

ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 11/03/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/522,405	COSSARIZZA, ANDREA	
	Examiner	Art Unit	
	Mark Staples	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-16* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-16* is/are rejected.

7)☒ Claim(s) *1* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *01/26/2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *09/30/2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Information Disclosure Statement

1. The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609.04(a) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered.

Specification

2. The disclosure is objected to because of the following informalities: "The Brief Description of the Drawings" found on p. 7 should be preceded by this section title. In addition, the brief description of the drawing on p. 7 refers to circles in Figures 1-4, however only diamonds and squares are found in these figures. Appropriate correction is required.

3. The use of the trademarks TEXAS RED™ and BLACKHOLE QUENCHER2™ has been noted in this application. They and any other trademarks should be capitalized wherever they appear and be accompanied by the generic terminology.

Although the use of trademarks is permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks.

Claim Objections

4. Claim 1 is objected to because of the following informalities: unclear grammar in step 1(d)(i). As recited in this step the repetition of the word "relative" is redundant and confusing. It is suggested one of the occurrences of the word "relative" be deleted and or the sentence be reconstructed to arrive at the intended meaning. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 1-16 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The term "CN" is used both as the copy number of NucSeqI in line 1 of claim 1 and as the relative copy number of NucSeqI in claim 1(d)(i). Thus it is unclear and indefinite as to what "CN" represents. The claims are further indefinite, since it is unclear as to how the ratio of the concentration of NucSeqI' and NucSeqII', as recited in lines 17 and 18 of claim, relate to the method of determining a copy number or a relative copy number.

6. Claims 1-16 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: constructing the standard curves

SC_I and SC_{II}. It is not recited what parameters of NuSeqI' and NuqSeqII' are known and it is not recited what measurements are made on NuSeqI' and NuqSeqII'.

7. Claims 1, 3-5, 8-9, and 11-16 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: determining the copy number of a first nucleotide sequence as recited in the preamble of claim 1. Claim 1 does recite a step for determining the relative copy number in claim 1(d) but claims 1, 3-5, 8-9, and 11-16 do not recite determining an absolute copy number.

8. Claims 2, 6, 7, 9, 10, 13, 14, and 16 recite the limitation "cell" in line 3 of claim 2. There is insufficient antecedent basis for this limitation in the claim.

9. Claims 7-9 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The recitation in claims 7-9 that NucSeqI and NucSeqI' are the same is in conflict with the base claim 1 recitation that NucSeqI' corresponds to NucSeqI. It is noted that the specifications defines “corresponding” as where one sequence is complementary to another sequence (see page 3 line 35 through page 4 line 8). Thus as recited in claim 1, NucSeqI' is complementary to NucSeqI; and hence the two are not the same and cannot be the same sequence. Thus it is unclear and hence indefinite as to what is being claimed in claims 7-9.

10. Claims 10-16 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which

applicant regards as the invention. The recitation in claims 10-16 that NucSeqII and NucSeqII' are the same is in conflict with the base claim 1 recitation that NucSeqII' corresponds to NucSeqII. It is noted that the specifications defines "corresponding" as where one sequence is complementary to another sequence (see page 3 line 35 through page 4 line 8). Thus as recited in claim 1, NucSeqII' is complementary to NucSeqII; and hence the two are not the same and cannot be the same sequence. Thus it is unclear and hence indefinite as to what is being claimed in claims 10-16.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

11. Claims 1-16 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 1-16 broadly recite a method of determining a copy number of a nucleotide sequence spectrophotometrically which includes colorometric, fluorescent, and luminescent measurements. However the specification only discloses fluorescent measurements with oligonucleotides that are labeled with a fluorophore. The claims do recite a method that determines a ratio which is not a relative copy number, as broadly claimed. Claim 2 and claims dependent from claim 2 also recite a method which uses

this ratio but the resulting determination is not an absolute copy number, as broadly claimed. The ratio and subsequent uses of the ratio would be accurate only under limited conditions and not as broadly claimed (discussed following and more below). Two Examples are given in the disclosure. Example 2 does not provide direction on how to determine a copy number (or relative copy number), but gives use of how copy numbers of a certain cellular oligonucleotide decrease in cells taken from people of increasing ages. In regards to Example 1, there is no direction on what is being measured and what would be done with those measurements. Thus, Example 1 does not provide sufficient direction on how to obtain a copy number or a relative copy number. Example I also refers to probes in order to use the invention. However the claims do no include the element of probes and do not recite a step using probes. Thus the claims as broadly recited, that is without the limitation of probes, are also not enabled. Due to lack of direction in the disclosure, one of skill in the art would have to perform undue experimentation to use the invention as broadly claimed. The claims are not enabled for either an absolute copy number or a relative copy number.

The lack of enablement for a relative copy number is further discussed.

The ratio, claimed to be a relative copy number, is determined by dividing Conc-I_{SCI} with Conc-II_{SCII} (see equation for "CN" in claim 1d). Conc- I_{SCI} is claimed to be the copy number of the first sequence, NucSeqI; and Conc-II_{SCII} is claimed to be the copy number of the second sequence, NucSeqII.

12. For colorometric measurements however, Conc- I_{SCI} can be biased, that is in error, due to the calculation of Conc- I_{SCI} by applying the colorometric measurement of

NucSeqI to a standard curve of NucSeqI'. As disclosed in the specification NucSeqI' is a sequence which is complementary to part or all (50% to 100%) of NucSeqI. Since NucSeqI' is complementary to NuqSeqI and NucSeqI' can be a different length than NuqSeqI; the standard curve of NucSeqI' does not give an accurate copy number for NuqSeqI. An accurate copy number for NuqSeqI will only be achieved in the limited circumstance where the extinction coefficient of NuqSeqI is equal to the extinction coefficient of NuqSeqI'. In other words, the same concentration of NuqSeqI and NuqSeqI' would have to give the same colorometirc measurement. The parallel analysis holds for Conc-II$_{SCII}$ as calculated by applying the measurement of NuqSeqII to the standard curve of NuqSeqII'. It is further noted that any bias in measurement of Conc-II$_{SCI}$ is independent of any bias in measurement of Conc-II$_{SCII}$. Since the biases need not be equal, the biases need not cancel each other out in the calculation of the ratio of Conc-I$_{SCI}$ divided by Conc-II$_{SCII}$. Thus the ratio can be biased and thus the ratio is not a relative copy number for a first nucleotide sequence, NuqSeqI.

In regards to NuqSeqI' being a different length than NucSeqI, Zhang et al.: ". . . it was known that the difference in the size of PCR products affects their optical density [colorometric measurement]. By using Ks [a size ratio constant], the potential error in calculating the number of mRNA copies has been corrected" (see page 774, 2nd column, 2nd paragraph).

13. For fluorescent measurements, Ginzinger et al. (2002) teach probes with fluorescent labels which function as follows as: (A) probe with a quencher and fluorophore where the quencher is cleaved, (B) a probe with quencher and fluorophore

which separate, or (C) a probe and a dye which binds only to the hybridized probe (see Figure 2). Example 1 of the specification does present a probe with a quencher and fluorophore but does not disclose how this probe functions.

Thus the specification of the instant application fails to disclose how biases in Conc- I_{scI} and Conc-II_{scII}, are corrected colorometrically or how fluorescent measurements are determined. Removal of any biases would be necessary to calculate a relative copy number or an absolute copy number. Due to the bias in measurements and in calculations of claims 1-16 and or absence of the essential element of labeled probes with the correct function, the claimed invention is not enabled. One of skill in the art would not know how to use the disclosed invention to determine a absolute copy number or a relative copy number of a first nucleotide sequence.

Claim Rejections - 35 USC § 102

14. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Due to the claim rejections given above, a distinction cannot be made between the claimed invention and prior art, as given in the following rejection.

Claims 1-3 and 6 are rejected under 35 U.S.C. 102(b) as being anticipated by Ginzinger et al. (2002).

Ginzinger et al. (2002) teach methods comprising the elements of claims 1-3 and 6. Ginzinger et al. (2002) teach additional elements of fluorescent detection, probes with fluorescent labels, and standard curve construction which result in methods that do determine an absolute copy number and do determine a relative copy number

Regarding claim 1, Ginzinger et al. (2002) teach a method of determining a ratio of a first nucleotide sequence comprising the steps of:

(1) adding to the sample nucleotides, primers, polymerase and optionally, any additional reagents, required for amplification (entire reference, especially Figures 1 and 2);

(2) performing one or more amplification cycles to amplify the target DNA (or mRNA);

wherein the sample comprises a chromosome-derived second nucleotide sequence, standard DNA, and the following amplification steps are carried out:

(a) target DNA is amplified,

(b) standard DNA is amplified,

(c) complements of target DNA and standard DNA are amplified (see sections p. 508, 1st column: "The unknown samples can simply be quantified by deriving the value from a standard curve generated with known samples from any of the three sources" and Figure 2 and the legend of Figure 4: "with about 5 or 6 bases of the 3□ end of one primer being complementary to the adjacent exon [target DNA]"); and

(d) standard DNA is present in a control sample and is amplified at multiple dilutions,

wherein amplification of the target DNA and standard DNA at multiple dilutions results in

the generation of standard curves such that a relative copy number can be determined from the fluorescent spectrophotmetric measurements applied to standard curves, and wherein the amplification reaction is performed in a single container (see p. 504, last sentence: "with up to three different PCR reactions in a single tube") and monitored spectrophotmetrically by fluorescence during amplification, and target DNA and standard DNA are localized on a single vector (entire reference, especially Figure 1 and Figure 2);

Claim Rejections - 35 USC § 103

15. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Due to the claim rejections given above, a distinction cannot be made between the claimed invention and prior art, as given in the following rejection.

Claims 1-3 and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al. (1997).

Zhang et al. (1997) teach methods comprising all the elements of claims 1-3 and 6, except Zhang et al. (1997) do not specifically teach methods constructing a standard curve with measurements of a sequence complementary to a standard sequence. But Zhang et al. (1997) do teach a method comprising additional elements not found in the

claims 1-3 and 6. Zhang et al. teach additional elements of correction factors and proper standard curve construction which result in methods that do determine an absolute copy number and do determine a relative copy number.

Regarding claim 1, Zhang et al. (1997) teach a method of determining a ratio of a first nucleotide sequence comprising the steps of:

(1) adding to the sample nucleotides, primers, polymerase and optionally, any additional reagents, required for amplification (entire reference, especially p. 771 and sections *Quantitative RT-PCR using various amounts of total RNA* and *Quantitative RT-PCR using various numbers of PCR cycles*);

(2) performing one or more amplification cycles to amplify the target RNA; wherein the sample comprises a chromosome-derived second nucleotide sequence, standard RNA, and the following amplification steps are carried out:

(a) target RNA is amplified,

(b) standard RNA is amplified,

(c) complements of target RNA and standard RNA are amplified (see sections *Quantitative RT-PCR using various amounts of total RNA* and *Quantitative RT-PCR using various numbers of PCR cycles*); and

(d) standard RNA is present in a control sample and is amplified at multiple dilutions, wherein amplification of the target RNA and standard RNA at multiple dilutions results in the generation of standard curves such that a relative copy number can be determined from the spectrophotmetric measurements applied to standard curves, and wherein the amplification reaction is performed in a single container (the same tube, see p. 770, last

sentence of 1st paragraph) and monitored spectrophotometrically during amplification, and target RNA and standard RNA are localized on a single vector (entire reference and especially Figure1);

Zhang et al. further (1997) teach a method of determining a relative copy number of a nucleotide sequence.

Notably, Zhang et al. (1997) teach as follows,

$D_t / D_s = (M_t S_t) / (M_s S_t)$ [1]

which is equivalent to:

$M_t / M_s = (D_t S_s) / (D_s S_t) =$ relative copy number [1a]

where

M_t is the [copy] number of molecules of target RNA,
M_s is the [copy] number of molecules of standard RNA,
D_t is the optical density of the target RNA,
D_s is the optical density of the standard RNA,
S_t is the size of target RNA, and
S_s is the size of standard RNA,

See page 772.

Regarding claim 2, Zhang et al. (1997) also teach a method wherein an absolute copy number is determined, per equation 4 below

$$M_m = (M_s K_s) / X_e \quad (4)$$

M_m is calculated and is the mRNA copies in 1 ng of total RNA, the copy number per ng,
M_s is known and is the [copy] number of molecules of standard RNA,
K_s is known and is the size ratio constant, ratio of the size of the standard RNA fragment to that of the target RNA,
X_e is determined from a prior standard curve prepared from known amounts of total RNA versus the ratio of optical densities of target RNA to standard RNA.

See page 772.

Regarding claim 3, Zhang et al. (1997) also teach a method wherein at least two different sequences used for measuring a corresponding number of different sequences are localized on a single vector (entire reference, especially examples for α, β, and γ fibrinogen genes and Figure 1 where standard RNA was synthesized from reverse-transcribed RNA with primers 1, 3, and 5).

Regarding claim 6, Zhang et al. (1997) also teach a method suing at least two different standard sequences on a single vector for measuring a corresponding number of different target sequences

Zhang et al. (1997) do teach PCR and the generation of complementary sequences in order to determine an absolute copy number and a relative copy number (entire reference, especially Figure 1). As noted above, Zhang et al. (1997) also do teach the construction of a standard curves using a standard sequence and the use of correction factors to arrive at accurate results.

Zhang et al. (1997) do not specifically teach the construction of a standard curve with measurements of a sequence complementary to a standard sequence in order to determine an absolute copy number and a relative copy number.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the teaching of Zhang et al. (1997) by constructing standard curves with measurements of a sequence complementary to a standard sequence, since Zhang et al. teach complementary sequences and teach that biases between measurements of different sequences can be rectified by correction

factors and proper standard curve construction. As suggested by Zhang et al. (1997) the standard curve can be constructed using measurements of a sequence complementary to a standard sequence with a reasonable expectation of success. The motivation to do so is provided by Zhang et al. (1997) who teach the usefulness of standard curves constructed with standard sequence measurements to measure target sequences. Thus, the claimed invention as a whole was *prima facie* obvious over the teachings of the prior art.

Reference of Record

16. Ginzinger et al. (2000) is made a reference of record as being of interest in the instant application. Ginzinger et al. (2000) teach: "Measurement of DNA Copy Number at Microsatellite Loci Using Quantitative PCR Analysis".

17. Zhang et al. (1999) is made a reference of record as being of interest in the instant application. Zhang et al. (1999) teach the design and use of primers for determining a copy number.

Conclusion

18. No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mark Staples whose telephone number is (571) 272-9053. The examiner can normally be reached on Monday through Friday, 9:00 a.m. to 6:00 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Mark Staples MS
Examiner
Art Unit 1637
October 26, 2006

KENNETH R. HORLICK, PH.D
PRIMARY EXAMINER

10/30/06